Studies on the Variation in Plant Nematodes

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CONTENTS

INTRODUCTION

The rapid growth of nematode taxonomy during the past three to four decades has created several problems and one is the realisation that variation exist at all the levels of classification (Thorne and Allen (1959). No doubt genetic variation lead to the formation of new taxa. Some of the morphological characters used in delimiting taxa in amongst the populations from a single host or single location may be expected to vary about 10-30% from the mean. Taylor and Jenkins (1957) on the basis of measurements of 100 females of Pratylenchus subpenetrans from a single host in one locality found that variation in length of females was to the tune of 20% of the mean, while Sher and Allen (1953) in P. vulnus concluded that the extent of variation was even more.

The ecological setting around the nematode populations including the host plant induce nongenetic variation (Malek and Jenkins, 1964; Gysels, 1964; Bird and Mai, 1967). Thorne and Allen (1959) reported that Ditylenchus destructor from sugar beets were smaller than those from potatoes, Considerable amount of work has since been done to estimate the extent of variability in amongst the population from different ecological settings. The literature has been extensively reviewed by Thorne and Allen

(1959); Bird (1967). The estimation of degree of variance is all the more important when dealing with quantitative or morphometrie and allometric characters. It actually determines which of the morphometric characters is most suited as taxonomic characters. In natural population the nematodes develop a kind of relationship with other components of the ecosystem and the chances of variability in amongst the population are very low. However, the chances of variation increases with the intensive agriculture practices leading to the development of new agroecosystem. Hence with this aim in view attempts will be made to study the following.

1. Effect of different plants both susceptible and resistant on the development of root knot caused by Meloidogyne incognita.
2. Effect of soil biota on the development of root knot caused by Meloidogyne spp. and on the morphometries of Meloidogyne spp.
3. Effect of fertilizers (N.P.K.) on the development and morphometrics of Meloidogyne spp.
4. Effect of different organic amendments on development and morphometrics of Meloidogyne spp.

REVIEW OF LITERATURE

Intraspecific variation or diversity has been reported in large number of phytoparasitic nematodes viz Aphelenchus avenae Bastian, 1865; Aphelenchoides saccheri Hooper, 1958; A. rutgersi Hooper and Myers, A. dactylocercus Hooper, 1958; Belonolaimus longicaudatus Rau, 1958; Cylindrocorpus crugii Goodey, 1939; Ditylenchus destructor Thorne, 1948; D. myceliophagus Goodey 1958(J); D. dipsaci Kuhn, 1857; Heterodera glycine Ichinohe, 1952; H. rostochiensis Wollenweber 1923; Helicotylenchus spp. Steiner, 1945; Hoplolaimus spp. Daday, 1905; Meloidogyne arenaria Neal, 1889; M. incognita (Kofoid and White 1919), Chitwood, 1949; M. hapla Chitwood, 1949; Neotylenchus spp Thorne, 1941; Panagrellus silusiae (de Man, 1913) Goodey, 1945; Paraphelenchus spp. Micoletzky, 1925; Paratylenchus nanus Cobb, 1923; Pratylenchus brachyurus Godfrey, 1929; P. vulnus Allen and Jensen 1951; P. subpenetrans Taylor and Jenkins, 1957; P. coffeae (Zimmerman 1898) Filipjev and Stekhoven, 1941; Radopholus similis (Cobb, 1893) Thorne, 1949; Rhabditis teres (Schneider 1866) Butschli, 1873; Rhodolaimus spp Fuchs, 1930; Bunonema Jagerskiold; Rotylenchus goodeyi Loof and Oostenbrink, 1958; Sectonema ventralis Thorne 1930; Trichodorus christie Allen, 1957; Tylenchorhynchus martinae Fielding, 1956; Xiphenema americanum Cobb, 1913; X. insignae Loos, 1949; and these variations can be broadly grouped into three main categories.

1. Genetic variation also known as congenital variation.
2. Physiological variation.
3. Morphological or phenotypic variation.

GENETIC VARIATION

In plant parasitic nematodes genetic variability may be induced by the influences of the host plant (Sturhan 1967). The most important source of genetic variation in population of sexually reproducing species is recombination while the mechanism in parathenogenetic population which develop on resistant varieties (Riggs & Winstead, 1959) is not clearly known. These variation may be expressed as morphological or physiological differences of various magnitude. However, some newly developing genetic variants are not readily detected in individual but can be detected as changes in one or more characteristic of the population. William et al., (1967) reported an unusual genetic variant in one of the populations of Panagrellus redivivus characterised by death of most individual, which was not observed in other cultures.

Variation due to mutation: Onions (1953) described four giant larvae derived from single cyst of H. rostochiensis and suggested that either gene mutation or polyploidy was responsible for their formation. Mulvey (1958) reported the production of giant larvae from second generation females of H. trifoli which had originated from a mixed population of this species and that of H. schachtii. Besides differences in body dimensions these giant larvae also showed anatomical differences. The stylet shaft was stouter and

knobs much heavier than normal larvae. The tail terminus was bluntly rounded and genital primordium was large. Netscher and Pernes (1971) observed differences in larval length of the offspring of two egg masses of H. oryzae.

Variation due to irradiation: Fyzzer and Honei (1961) and Babero (1952) obtained specimens of Panagrellus selusiae which were bigger in size as a result of Gamma and X ray irradiations while Could, Gomberg, Bothell, Viltella and Hertz (1955) reported reduction in the size. Myers (1960) reported thickening and nodulation of the cuticle in larvae of Tylenchorhynchus sp. Siddiqui and Viglierchio (1970) obtained an aberrant form of D. dipsaci which resembled the rhabditoid mutant of D. dipsaci isolated by Sturhan (1967) from Cucumber and melon cotyledons. In such specimens the posterior two third of the body was swollen terminating in a dome shape tail. Larvae and adults showed thickly cuticularized stylet, Oesophageal lumen, excretory canal and vaginal wall.

Variation due to resistant plants: The fact that a population acquires the ability to attack resistant host indicates that some genetic variation has occurred either within the pathogen or in the plant itself (Haggoner and Wallin, 1952). Ross, (1958) obtained a population of the soybean cyst nematode capable of attacking

soybean variety 'peking' resistant to the nematode. Jones (1957) Dunnet (1957), Huijsman (1957), Vanderlannand Huijsman (1957), Goffert (1957) reported that as a result of inoculation a population of H. rostochiensis developed which formed mature females on resistant potatoes. This later led to the concept of pathotypes in this nematode which were ~~later~~ numbered as A,B,C,D,E. Webley (1970) measured larvae of several British populations of H. rostochiensis and found that those of pathotypes A were shorter, had shorter stylet and a shorter distance between the median bulb and excretorypore as compared to those of pathotypes B or L. Trudgill, Parrot and Stone (1970) also observed differences between males of different pathotypes. Green (1971) reported differences in the vulval region of females and Stone (1972) in lips and oval discs of second stage juveniles. These differences together with other evidences led to the description of new species Globodera pallida Stone 1973. Stone (1973) reported that continuous growing of potato resistant to biotype A of H. rostochiensis changed a population from almost entirely biotype A to largely biotype B. Riggs and Winstead (1959) maintained a population of M. incognita incognita, M. incognita acrita, and M. arenaria arenaria on resistant tomato Hawaii 522 for many generations and obtained morphological differences between the perineal pattern of the so developed new strains and the parent population. These new strains had the inherent

genetic ability to develop in presence of Mi genes. Gjimalva, Martin and Hernandez (1963) obtained an isolate of M. incognita which was able to reproduce on vart Heartgold a resistant variety of sweet potato.

PHYSIOLOGICAL VARIATION

Variation in pathogenecity was first reported by Ritzema Bosa (1888) and since then physiological variation has been reported in large number of phytoparasitic nematodes.

The physiologic variation may manifest themselves as:

1. Variation in host range i.e. ability to attack different plant species and varieties and to multiply on them.

2. Variation in response to plant attractants.

3. Variation in penetration in plants as reported by Baines et al., (1967) for Tylenchus semipenetrans and Sturhan (1968) for D. dipsaci.

4. Variation in expression of symptoms. Koketal (1963) reported that different symptoms were produced on tulip when attacked by tulip and onion races of D. dipsaci. Similar results were obtained by Howell (1966) and Windrich (1970) with tulip and narcissus race of D. dipsaci and Epps and Golden (1967) in H. glycine on soybeans.

5. Variation in reproduction rate, speed of development and degree of infestation on same host has been reported in M. incognita and M. arenaria. (Minton 1963, Lasser 1966); H. rostochiensis (Kort 1966); T. christie (Bird and Mai 1967); D. dipsaci (Sturhan 1969); Tylenchus semipenetrans (Baines et al., 1969). Physiological variations between populations of different geographical origin and also among populations of a single biological race have been reported in large number of nematodes (Seinhorst 1957, Southey 1957, Epps and Golden 1967, Allen 1952, Martin 1954, Riggs and Winsted 1959).

Intraspecific variations: Although biotypes in D. dipsaci were reported by Hodson (1926) Smith (1951), Seinhorst (1957) and Southey (1957) but Seinhorst (1957) suggested differential hosts for distinguishing elven biotypes in these nematodes. No morphological differences were found between alfalfa and teasel strains but Hasting et al., (1952) and Smart and Darling (1963) reported differences in pathogenecity among populations of D. destructor derived from different hosts. Similar results were obtained by S'Jacob (1962) for D. radicicola (Grof) Vander Laan. Huijsman (1957), S'Dunnet (1957) reported a race of H. rostochiensis capable of reproducing on Solanum andigenum and on S. tuberosum hybrids. Halstead and Bingefors (1963), Kort et al., (1967), Cotton (1967),

Neuber (1967) reported the existence of races in H. avenae (Woll), Raski (1952), Shepherd (1959) in H. schachtii (Shmidt), Ross and Brim (1957); Apps and Golden (1967), Miller (1965) in H. glycine. Merny (1968) reported two races in H. humuli viz. hop race and ficus race.

Variation in pathogenecity among the populations of Meloidogyne is not uncommon. Christie and Albin (1944) demonstrated the existence of races within the former species of H. marioni which actually formed the basis on which Chitwood (1949) reclassified it into the genus Meloidogyne. Martin (1954) found variation in the pathogenecity of M. incognita and M. incognita Var acrita on different cultivators of cotton. Colbran (1958) observed distinct physiological races in M. arenaria (Neal) Chitwood, M. hapla Chitwood, M. incognita and M. javanica (Treub and Chitwood). Ciamalva, Davide and Struble (1963) found similar situation with M. incognita on sweet potatoes. Goplen (1950) while testing the pathogenicity of 20 collections of root knot nematode on five alfalfa cultivars found 3 biotypes of M. incognita Var acrita and 2 each of M. javanica and M. hapla. Sasser (1963, 66) while working with world wide collections of Meloidogyne spp. on nine host differential reported the existence of several physiological races in M. incognita and M. arenaria. Trantaphyllou and Sasser (1960) described variation both in perineal patterns of females from different host and host specificity of M. incognita. Riggs and

Winstead (1959) reported that certain new strains of M. incognita developed in green house which were capable of attacking resistant tomato plants. Graham (1969) discovered a new race of M. incognita in field plots of flue cured tobacco which attacked NC 95 tobacco, a cultivar resistant to M. incognita. Ogbiyl and Jensen (1972) reported the existence of five biotypes in M. hapla. Southards and Priest (1973) on the basis of diversity in seventeen isolates of M. incognita, reported six physiological races on six hosts. Mechill, Malek, Taylor and Edward (1973) demonstrated the existence of atleast five physiological races of M. nassi which could be differentiated by their reactions on Torontoc 15, creeping bent grass, curly dock and RS-610 Sorghum. Kirby, Dickson and Smart (1975) reported considerable intraspecific variation in host preference in amongst 25 populations of Meloidogyne species.

Existence of physiological races in Pratylenchus penetrans has been reported by Slootweg (1956); Olthof (1968) in P. neglectus, Jensch and Loof (1960) in P. loosi Loof, and B. longicaudatus by Perry and Smart, in T. semipenetrans by Baines (1967) and Radopholus similis by Du Charme and Birchfield (1956). In R. similis two races have been described i.e. the "banana race" attacking banana, but not citrus and the "citrus race" attacking both banana and citrus. Burckhardt (unpublished) observed variation in virulence of Aphelenchoides fragariae, A. ritzemabosi from different hosts and

different localities. Van Hoof (1967) investigating transmission of black ring Virus by several Dutch populations of Longidorus elongatus demonstrated that population may differ in their ability to transmit virus. He further (1968) reported that certain populations of Trichodorus plachydermus were specific for transmitting certain isolates of tobacco rattle virus.

Interspecific variation: Considerable amount of variation in amongst the different individuals of single egg mass isolates have been reported in M. incognita by Allen (1952) Martin (1954) and Triantaphyllou and Sasser (1960). Riggs and Winstead (1959) reported the selection of new "B" biotypes causing severe root knot galling on tomato plants resistant to "A" biotype of green house cultures of M. incognita and M. arenaria. Sturhan (1965) observed variation in host range between different pure cultures of D. dipsaci each obtained from one fertilized female of the same field population. Sturhan (1966) further obtained spontaneously high rate of reproduction of red clover strain of D. dipsaci on sugar beet but could not obtain the same results with subsequent inoculations. Furthermore in some cases he found that stem eelworm larvae, unexpectedly did not reach maturity in host plants of the pure strains form which they originated, despite being present and apparently feeding there for more than three months (Sturhan 1968). Miller (1966) reported that two isolates from single cysts of

H. glycines differed greatly in the development of cysts of "Lee" soybean. Kort (1966) found that in H. rostochiensis the eggs inside the cyst were heterogenous and suggested that this might be the probable reason for variation in this species. Later Howard (1968) obtained two inbreeding lines with different fecundity from single cyst of potato root eelworm.

MORPHOLOGICAL VARIATION

Morphological characters used in nematode taxonomy vary considerably among and within a nematode population. Taylor and Jenkins (1957) working with Pratylenchus and Coomans (1962) with Rotylenchus goodeyi found great diversity in the population and the deviation from the mean was lowest for the character of Vulva position and greatest for the tail shape. Although Godfrey (1929) reported variation in tail shape in P. brachyurus from a rounded to a square terminus but Taylor and Jenkins (1957) confirmed it in four other species of Pratylenchus. Tarte and Mai (1976) reported two different variations in tail terminus of females of P. penetrans Cobb i.e. smooth and crenate tailed, the later having termini with two or more annules. Variation in tail shape has also been reported in the soybean cyst nematode by Golden and Epps (1965) in Hemicycliophora zuckermani by Minton (1965). Hollis and Whitlock (1959) reported an abnormal variant in Tylenchorhynchus martinae

and T. ewingi with dorsal hump and bifid tails of unknown causation. Heihm (1965) demonstrated great variation in the structre of cuticle and parts in species of the subgenus Rhodolaimus living in mulm of Araucaria raucana. This variability was also studied in populations from fresh mulm, old mulm and old mulm suspended in water. One and the same population gave a range from waterless individuals to very warty ones. The specimens which developed later had unpaired rows of wart also. The number of different variations developing depended on age of the sample and also on the fact whether they were suspended in water or not. Similarly, variation was observed in the position of the vulva, shape of the stylet knobs, position of the phasmid, excretory pore, number of annules number of ventro median supplements (Ching 1969, Coomans 1962, 63, Deconinck 1962, Sher and Allen 1953). Bajaj and Jairajpuri (1977) found that twenty three populations of X. insignae from India showed extreme variability within the species and divided them into two groups (a) insignae form, (b) indicum form. The adults and juviniles of the two forms differed from each other in the shape of the lip region, length of odontostylet, position of the fixed guiding ring from anterior extremity, tail length and c/ values and ratios. Fisher (1965) during counts of population of nematode under different environmental conditions noticed that there was considerable variation in length of the female and on some occassion

a complete mixture of all length occurred in one population where as in some there was predominance of either shorter and longer nematodes.

These variations have been attributed to be due to multiplicity of factors most of which are environmental. Of the different factors host plant, host nutrition, soil type, soil temperature, nematode density and diverse geographical localities have been playing a significant role in the origin of variation in the morphometric characters.

Effect of host: Phytoparasitic nematodes feed on plant roots and their aerial parts and also on soil micro-organisms. Hence all these groups of organism have been known to bring about variation in nematode morphology and dimensions.

One of the outstanding example of host induced variation is that of *D. destructor* reported by Barker (as cited by Thorne, 1959) where the specimens from sugar beet were smaller in size and oesophageal lobe dorsal in position on the contrary the specimens from potato were larger and had ventral overlap. He also reported the change of the host altered the characters also. Christie (1936) reported that nutritional effects in the form of favourable or unfavourable host may exert a profound change in body size and gonad development. He found that in *Agamermis decaudata* the female ranged in size from

three to fourty six and a half centimeter and that small females produced small eggs. Ludwig (1938) while studying variation in Rhabditis teres found that alteration of the various constituent of the medium caused changes in certain morphological feature and dimensional ratios of different organs. Some of the changes were striking, for instance the spicule length was increased considerably when certain plant juices were added to the medium. This was later confirmed by Stephenson (1942). T. Goodey (1941) reported that a giant race of D. dipsaci from broad beans contained larger females as compared to that of the oat race and that the oat race did not increase in size when reared on broad beans. Gemmell (1943) reported that inoculation of different potato varieties Epicure and Doonstar with H. rostochiensis resulted in the development of smaller cysts whereas when Golden Wonder and Majestic were inoculated the cysts were larger J. Goodey (1952) reported host induced variation in D. destructor, Van Meerdt (1958) in Radopholus similis; Barraclough and Blackith (1962) in D. dipsaci D. myceliophagus Bird and Mai (1965, 66) in T. christie, Brzeski (1961) in D. myceliophagous; Behrens (1974) in H. pallida; Cook (1975) in H. avenae. Tacioni (1969) and Pogosyan (1976) reported variation in vulval plate in M. hapla, M. arenaria and M. incognita obtained from different hosts. Trudgill et al., (1970) observed that resistant hosts often decreased the mean body length and stylet length in males and larvae

of H. rostochiensis. They further reported that physiological status of the host also influenced these features. Heihm (1962), Sanwal (1965); Evans and Fisher (1970); Elsherif (1972). Hooper and Myers (1971) reported that specimens of Aphelenchoides rutgersi varied greatly according to the type and age of cultures of fungi. They also reported that there was no consistency in many of these characters. Grulton, Dickson and Esser (1976) found morphological and morphometric differences amongst three single egg mass population of M. arenaria when reared on different host. Chirr (1977) reported that host organism on which Aphelenchoides fragariae and A. besseyi were raised influenced the absolute values and variability of different characters measured. However, Hasbrouch and Jenkins (1960) observed no apparent host influence on the variability of different morphological characters in T. claytoni. Tyler (1933) reported that number of eggs laid, size and vigour of the larvae were affected by the nutritional status of the host. Cover, Smart and Darling (1965) found that body length of Ditylenchus species was similar at all the nutrient levels except at low and zero potassium and zero phosphorus where they were shorter. Sanwal (1965) suggested the nutrition of the host plant might influence the size of the Ovary in Aphelenchoides spp. Rashid and Khan (1976) found that body length in P. coffeae obtained from roots were larger than those from soil. Ismail and Saxena (1977) observed that higher potassium levels favoured the growth of M. incognita at all stage

of the development and cross sectional area of both larvae and female of M. incognita increased with the increase in potassium supply to the plant.

Similarly, different fungal food sources brought about changes in morphometrics of nematode. Pillai and Taylor (1967) reported that morphometrics of different characters in a population varied when reared on different fungi. Myers (1967) noted that there was considerable amount of variation in the body size of the females as a result of growing on different culture media. Monson (1971), Townshed and Blackith (1975) reported that type and age of cultures also affected the nematode morphometrics. Royand Dunn (1975) on the other hand reported that A. avenae multiplying on two different fungus cultures showed no significant differences in any of the morphometric characters. Oteifa (1953) observed no change in the size of the females of M. incognita when host plants were supplied with different concentrations of potassium. Similarly, certain bacteria which constituted the food source influenced the taxonomic features of Rhabdites briggsae (Nigon and Dougherty 1950); Acrobeloides uberrinus (Anderson 1965) Acrobeloides spp. (Anderson 1968); A. nanus (Sohlenuis 1973). Anderson (1965) reported that certain diagnostic features of an Acroleloides species specifically the shape of the labial probolae and tail varied significantly when grown on bacterial cultures as compared to those in soil. Kiesiel et al., (1969) reported that nematodes cultured with bacterial

growth factor differed from those maintained in liver extract by the development of prominent swellings immediately posterior to anal openings.

Effect of temperature: According to the "rule of Bergmann" the smaller species of any group of vertebrates will be found in warm regions and the larger in the colder region. The fact that this is also applicable to nematode was shown by De Coninck (1935) in Dorylaimus thermae (Thorne 1936), in Trichodorus spp. by Rhode and Jenkins (1957); Panagrellus silusiae deMan (1964); T. christie. Malek and Jenkins (1964); Paratylenchus nanus Fisher (1965) Garaert (1965); Xiphinema sp. Tarjan (1969), H. rostochiensis, M. hapla Wong and Mai (1973). However, Evans and Franco (1977) reported that high soil temperature increased nematode size in Globodera rostochiensis and G. pallida. Wong and Mai (1973) on the other hand reported abnormal males in M. hapla and M. javanica at 35°C. Malek, Jenkins and Power (1965) found however no correlation between the temperatures and the total length, width and stylet characters in Criconemoides curvatum Raski (1952) on Vicia villosa (hairy vetch) and carnations except for slight smaller size of nematode at 30°C on carnations.

Effect of population density: That high population density (resulting in paucity of space) resulting in reduction in the size

has been reported in Paratylenchus nanus by Fisher (1965); in D. myceliophagous by Evans and Fisher (1970), in H. schachtii by Keretan (1971); in H. glycine by Evans and Franco (1977).

Geographical factors: Bird (1966) and Bird and Mai (1967); Tarjan (1969); Heyns and Von Ark (1973), and Lamberti (1973) pointed out that population of nematodes from different geographical areas exhibited morphological and morphometric variation.

The differences in the population of Longidorus africanus Merny obtained from different geographical regions lead Lamberti (1973) to divide the species into three groups viz (1). The East African type from Rhodesia and Somalia land, measuring 2.8-3.1 mm in length. (2). The North African type from Egypt, Sudan and Israel measuring 4.0-4.5 mm and (3). The California type and intermediate type measuring 3.5-3.9 mm.

Yeats (1972) reported that the various dimensions of Tylenchus leptosoma; Plectus parietinus, Bastian 1865, Tylencholaimus mirabilis in a given locality remained uneffected. Besides population derived from a single female from different localities has been shown to exhibit a high degree of variability. (Van weerdt 1958; Sanwal and Loof 1967 , Tarjan and Fedrick 1974, and Chu 1975). Allen (1952) while comparing the perineal patterns of M. hapla with their progenies indicated that the female does not produce progeny

exhibiting all characters of parent. Females with lateral extension of the pattern produced progenies lacking this extension. Likewise females having rather marked lateral lines produced progeny that did not exhibit any evidence of these lines. Moreover, the number of labial annules in males also varied from zero to three.

It is thus evident from the above that environmental factors do bring about variation in morphological characters and this aspect has little been studied. This is more true with respect to root knot nematode, Meloidogyne incognita. Hence systematic study on the extent of variation induced by different environmental factors on root knot nematode will be taken up.

MATERIAL AND METHODS

Roots infected with Meloidogyne incognita will be collected from different hosts and localities to raise single egg mass population. The females of the nematodes and perineal region will be studied to ascertain the species of Meloidogyne. As far as possible the seedlings of the same plant from which single egg mass has been collected will be inoculated under laboratory conditions in pots. Where such host seedlings are not available tomato CV Marglobe will be inoculated for raising cultures. Seedlings of the host plant will be raised in autoclaved soil. Single egg mass will be transferred near the roots by removing the upper soil. Seedlings will be subsequently inoculated with these cultures in order to have a regular supply of the inoculum.

Effect of host plants: Different vegetables, pulses and ornamentals will be tested for their suitability and the extent of variations that they induce. The seeds after having them surface sterilised with $HgCl_2$ and rinsed with water will be sown in sterilised sand-soil mixture (1:3). The seedlings when in 3-leaf stage will be inoculated with 1000 larvae. After 30 days of inoculation the plants will be uprooted and nematodes will be killed and fixed for morphometric studies. The development of root knot nematode will be determined. Plants on which significant variation

in characters of nematode has been observed will be selected for further study. On these plants the nematode will be reared for three generations by making inoculation of fresh seedlings each time after 30 days. The probable period of completion of one generation in plant. The inoculum for subsequent inoculation of plants will be taken from the infected roots of the preceding generation. After completion of each interval of 30 days the nematode will be examined for various morphometric and allometric characters.

Effect of soil biota: Plant most susceptible to root knot nematode in the above categories will be selected for studying the effect of soil biota on the variation in morphometrics. The plants grown both in naturally infested soil and autoclaved soil will be inoculated with 1000 larvae of M. incognita per 500 g soil/ plant. The nematode will be allowed to remain in these host for 3-5 generations by making repeated inoculations on the host plant each after an interval of 30 days. Each time after 30 days the plants will be uprooted and growth will be determined. Each time the nematodes will be killed and fixed and morphometric studies will be made. The population of nematodes and the rhizosphere fungi will also be determined. The nematodes after three generations in unautoclaved soil will be used to inoculate the plants of the same host in sterilised soil. The extent of variation in different morphometrics and allometric characters will be determined.

For the isolation of the rhizosphere fungi Warcup's (1950) plate method will be used. The soil around the roots of plants will be brought to the laboratory in sterile containers. The plants will be shaken to remove superfluous soil from the root system. The soil adhering to the roots will be collected and a little amount of soil with the help of sterilized flattened tip of a needle will be transferred to petridishes containing 10 ml of sterilized molted and cooled peptone dextrose agar medium. (Parkinson, 1957).

Agar	20 gm
KH_2PO_4	1.0 gm
$MgSO_4.7H_2O$	0.5 gm
Peptone	5.0 gm
Dextrose	10.0 gm
Distilled water...	1000.0 ml
Rose Bengal.....	1:30,000
Streptomycin.....	3/ug/ml
or	
Aureomycin	2 ug / ml.

(Martin, 1950) (Johnson 1957)

The petriplates will be rotated before the solidification of agar in order to disperse the soil particles evenly, and will be

incubated at 28°C. The fungi which will develop after one week of incubation will be examined and identified. The frequency of fungi will be calculated as follows-

$$\frac{\text{Number of plates containing a particular fungus}}{\text{Total plates poured}} \times 100$$

In order to determine the population of fungi the average weight of the soil held on a flattened tip of needle will be determined. The number of colonies developed in all plates will be counted and this figures will be transformed to the number of colonies per gram of soil.

For determining the relative abundance of the fungi the formula suggested by Mclean and Cool (1957) will be employed.

$$\frac{\text{Total no. of colonies of fungus}}{\text{Total no. of colonies of all fungi}} \times 100$$

Effect of N.P.K. fertilizers: Since there has been a tendency to use indiscriminating inorganic fertilizer for growing crop, it was considered desirable to study the extent of variation induced by them. The different fertilizers (N.P.K.) will be added at the following rate:

N = 216 mg/ kg of soil

P = 160 mg/ kg of soil

K = 52 mg/ kg of soil

Treatment I (NPK alone)

Nitrogen as Ammonium sulphate = 216 mg/kg of soil

Phosphorus as Superphosphate = 160 mg/kg of soil

Potassium as Potassium nitrate = 52 mg/kg of soil

Treatment II

N + P	Nitrogen and Phosphorus	=	(216 mg of Ammonium sulphate + 160 mg of super phosphate)
N + K	Nitrogen and potassium	=	(216 mg of Ammonium sulphate + 52 mg of Potassium nitrate)
P + K	Phosphorus and potassium	=	(160 mg of super phosphate + 52 mg of potassium nitrate)
N+P+K full dose	Nitrogen, phosphorus and potassium	=	(216 mg of ammonium sulphate + 160 mg of super phosphate + 52 mg of potassium nitrate)
Half dose	Nitrogen, phosphorus and potassium	=	(108 mg of ammonium sulphate + 80 mg of super phosphate + 20 mg of potassium nitrate)
Double dose	Nitrogen, phosphorus and potassium	=	(432 mg of ammonium sulphate + 320 mg of super phosphate + 104 mg of potassium nitrate)

Tomato seedlings will be transplanted in soil containing the above mentioned doses of fertilizers and will be inoculated with root knot nematode. After each generation till the third generation the nematodes will collected and killed and fixed and morphometric studies will be made. The growth of plant and number of nematodes will also be determined at the termination of experiment.

Effect of different organic amendments: Tomato seedlings will be transplanted in the soil amended with different oil cakes viz. Castor (Ricinus communis L), Mustard (Brassica campestris L), Neem (Azadirachta indica Juss), Mahua (Madhuca indica Gmel) and Groundnut (Arachis hypogaea L) at the rate of 1 g N per kg soil. Ammonium sulphate, potassium nitrate and Superphosphate and compost (Cow dung manure) will be added to the soil at the same rate for control. The seedlings will be inoculated with root knot nematode. After every generation upto three generations the nematodes will be killed and fixed and morphometric studies will be made. Later egg masses from plants grown in soil amended with oil cakes will be transferred to plants grown in soil amended with fertilizers and compost manure inorder to determine whether these changes are reversible. The growth of plant and number of nematodes and rhizosphere fungi will be determined at the termination of experiment.

Preparation of slides: Roots of infected plant will be chopped into small pieces, and will be killed and fixed by adding warm cotton blue in lactophenol. The material will be kept in lactophenol till it is cleared. The nematodes in their different stages of development will be dissected and mounted in lactophenol in the cavity slides whereas the second stage larvae and males will be mounted in a drop of lactophenol on glass slide and covered with the cover glass with the support of 3 pieces of glass wool. The perineal region of the mature females will be mounted on slide in lactophenol for examination.

Record of data: After 30 days the plants will be uprooted and length, fresh and dry weight of plants will be recorded. Root knot index will be rated as follows:

0 = No evidence of Infection

1 = Infection but larvae do not mature into adult females

2 = Few larvae develop into mature females but do not lay eggs.

3 = Females with egg masses 1-20 per plant roots moderately galled

4 = Females with egg masses 20-40 per plant roots moderately to severly galled

5 = Females with abundant egg masses. Severly galled.

The final population of nematodes both in the roots and soil will be determined. Isolation of nematodes from soil will be made by using Cobb's sieving and decantation method and from roots by Blender method (1953). Five gms root from each treatment will be blended in a waring blender which will be operated for 30 seconds. The nematode suspension obtained from 250 gm of soil or 5 gm of roots will be kept on tissue paper mounted on coarse sieve. The whole assembly will be kept in an enamel tray. After 24 hours the aliquotes will then be transferred into a beaker and 5 ml from this will be counted under stereoscopic microscope. Three such countings will be made, and the figure thus obtained will be multiplied to obtain the values per 250 g soil/ 5 gm of roots.

The following characters of Meloidogyne females males and larvae will be taken into consideration for morphometrics.

Female

1. Body length
2. Body width
3. Neck length
4. Neck width
5. Position of excretory pore
6. Stylet length
7. Knob width
8. Opening of dorsad; oesophageal gland
9. Median bulb length and width
10. Median bulb valve length and width

Perineal pattern

1. Length of Dorsal arch
2. Length of ventral arch
3. Vulval width
4. Vulval anal distance
5. No. of lines on the right side of pattern
6. No. of lines on the left side of pattern
7. Presence or absence of lateral field
8. Presence or absence of punctation.

Larvae

1. Body length
2. Body width
3. Tail length
4. Stylet length
5. Knob width
6. Median bulb length
7. Median bulb width
8. a = length divided by greatest body width
9. c = length divided by tail length
10. d = tail length divided by body width
11. L/ℓ_1 = length divided by body length from anterior end to posterior end of oesophagus
12. L/ℓ_2 = length divided by body length from anterior end to middle of the median bulb.

Male

1. Body length
2. Body width
3. Stylet length
4. Knob width
5. D.G.O. Orifice
6. Head length
7. Median bulb length

8. Median bulb width
9. Median bulb valve length
10. Median bulb valve width
11. Spicules (length of arc)
12. Guber-naculum
13. a,c and d values will be determined.

Eggs: Length and width of unembryonated eggs.

The data so obtained will be subjected to statistical analysis to determine the Co-variance ratio etc.

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